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博士論文（要約）

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Monoacylglycerol lipase (MAGL)を標的としたPETトレーサー [^{18}F]**T-401**の開発

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The clinical cost per approved new drug has increased more than 10 times in 20 years and was estimated to be $ 1,460 million in the 2000s. In addition, the success rate of therapeutic drugs targeting disorders of the central nervous system (CNS) is low, less than 10% mainly because of the lack of animal models reflecting the pathology of CNS disease in human. An estimation of effective dose of drugs, selection of appropriate patients based on mechanism of action and efficacy prediction in short-term trials by biomarkers expected to be potential options to increase the success rates of clinical trials for CNS-targeting drugs.

Positron-emission tomography (PET) is a non-invasive imaging technology that allows quantification of physiological and pathological processes in living subjects. PET imaging could potentially provide a means to determine the occupancy of a target molecule in human brain by therapeutic inhibitors. This information obtained from *in vivo* PET scans can afford dose setting in clinical trials, which could lead to rational protocol design and following go/no-go decision. In addition, PET imaging could allow the patient stratification for the clinical trial and the efficacy evaluation of the drug by using the radiotracer as a diagnostic marker and a surrogate marker for efficacy, respectively. Given these factors, PET technology is expected to increase success rate of the clinical trial and save its cost. Because the above technologies require a PET radiotracer that binds a target molecule specifically, the development of radiotracers is also highly required.

Endocannabinoid neurotransmission in the central and peripheral nervous systems is dependent on cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) and their endogenous fatty acid ligands. The primary endogenous ligands for CB1 and CB2 receptors are N-arachidonoyl ethanolamine (AEA), also known as anandamide, and 2-arachidonoylglycerol (2-AG). Both regulate multiple physiological processes in CNS, including pain, inflammation, appetite, memory, and emotion. AEA and 2-AG are biosynthesized by the phospholipase-catalyzed hydroxylation of membrane phospholipids and are rapidly inactivated by enzymatic hydrolysis after exerting physiological actions through the cannabinoid receptors. The degradation of these endocannabinoids is mainly mediated by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL).

MAGL is a cytosolic serine hydrolase that cleaves monoacylglycerols into fatty acids and glycerol. It is responsible for about85% of 2-AG hydrolysis. Thus, inhibition of MAGL increases 2-AG levels, leading to activation of endocannabinoid neurotransmission and consequent anti-nociceptive, anxiolytic, and anti-emetic responses. In addition, MAGL inhibition decreases arachidonic acid levels, resulting in anti-inflammatory and neuroprotection effects in the brain. Thus, MAGL could be a promising target for the treatment of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease and neuropsychiatric disorders in which the endocannabinoid system and neuroinflammation have mechanistically been implicated.

Several PET imaging agents for MAGL have been reported, and some of these compounds exhibit selectivity for MAGL and high uptake in the brains of rodents and non-human primates. However, all the agents reported bind to MAGL irreversibly, preventing quantification of binding components. Accordingly, I aimed to develop PET imaging agents that bind to MAGL selectively and reversibly.

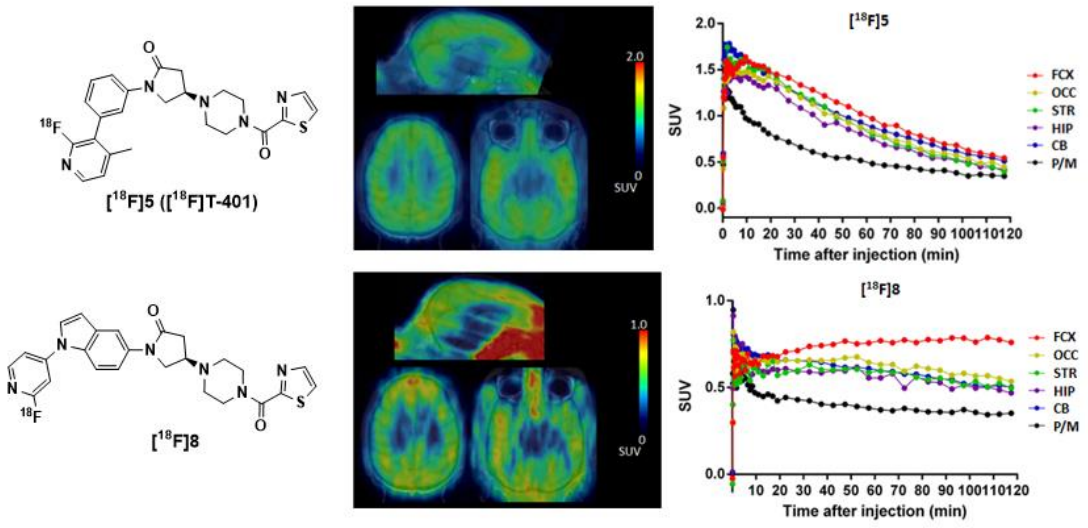
In this thesis, I present the development of novel PET imaging agents that bind to MAGL selectively and reversibly in chapter 2 and PET studies using the optimized MAGL radiotracer, [^{18}F]**T-401**, for quantification of MAGL in the brain of rhesus monkey and its occupancy by a well-known MAGL inhibitor in chapter 3.

CHAPTER 2: Design, Synthesis, and Evaluation of Novel Positron-Emission Tomography Imaging Agents for Monoacylglycerol Lipase (MAGL)[1]

Central nervous system PET imaging agents need not only high affinity and selectivity for the target in order to provide sufficient in vivo binding potential, but also desirable physicochemical and pharmacokinetic properties that allow efficient blood-brain barrier (BBB) penetration, low levels of undesirable nonspecific binding (NSB) and low level of brain penetrant radiometabolites. Considering these factors, I selected the recently identified piperazinyl pyrrolidin-2-one derivative **1** which binds to MAGL reversibly as a lead compound for the development of a new class of MAGL PET imaging agents. By tailoring the lipophilicity of the molecule to optimize nonspecific binding and blood-brain barrier permeability, I successfully identified two compounds (**5** and **8**) that show high uptake to regions enriched with MAGL in the wild-type (WT) mouse brain in *ex vivo* LC-MS/MS studies using unlabeled **5** and **8**. The uptakes of these compounds were reduced in MAGL-knockout (KO) mice, demonstrating that the binding of compounds **5** and **8** in the mouse brain is highly specific to MAGL.

[^{18}F]**5** and [^{18}F]**8** were synthesized by [^{18}F]fluorination of the precursors with high radiochemical purity (>99%), and their radiochemical yields decay-corrected for cyclotron-produced [^{18}F]fluoride were 21.7 ± 3.0% (n = 11) and 20.1 ± 4.6% (n = 3), respectively. Their molar activities were 391 ± 99 GBq/μmol for [^{18}F]**5** and 191 ± 67 GBq/μmol for [^{18}F]**8** at the end of the synthesis.

PET imaging of WT and MAGL-KO mice revealed that the highest uptake of [^{18}F]**5** and [^{18}F]**8** occurs in the frontal cortex, followed by the striatum and hippocampus, and the lowest radioactivity uptake is that in the midbrain. The uptakes of [^{18}F]**5** in all these regions peaks immediately after injection, and decreases to 10–20% of the maximum at 90 min. The kinetics of [^{18}F]**8** in the brain are slower than those of [^{18}F]**5**, with its uptakes in the frontal cortex, hippocampus, and striatum peaking at ≈15 min, followed by a gradual decline in radioactivity to 40–60% of the maximum at 90 min. The differences in the pharmacokinetic properties of [^{18}F]**5** and [^{18}F]**8** in the mouse brain are consistent with the results of LC-MS/MS study, validating this *ex vivo* assay as a means for predicting *in vivo* profiles of compounds. In MAGL-KO mice, the uptakes of [^{18}F]**5** and [^{18}F]**8** were profoundly reduced compared with those for WT mice, and the radioactivity distribution is fairly uniform across these brain regions. In a rhesus monkey, [^{18}F]**5** (named [^{18}F]**T-401**) showed adequate kinetics for PET quantification of MAGL, whereas the kinetics of [^{18}F]**8** in the brain were slower than those of [^{18}F]**5**, and the radioactivity uptake in the frontal cortex did not reach pseudo-equilibrium within the 2-hour PET imaging session.



CHAPTER 3: Quantification of MAGL and its Occupancy by an Exogenous Ligand in Rhesus Monkey Brains using [^{18}F]T-401 and PET

I have developed [^{18}F]**T-401** as a novel and selective PET imaging agent for MAGL in chapter 2, and reversible binding properties of this radiotracer is suitable for quantitative assays of MAGL. In this chapter, I aimed at determining an analytical method to quantify MAGL in the brain of rhesus monkey and its occupancy by an exogenous inhibitor JW642 by PET using [^{18}F]**T-401**.

In a rhesus monkey, [^{18}F]**T-401** efficiently entered the brain with its uptake peaking at 5-15 min followed by a continuous washout from all brain regions over 120 min. The highest retention of [^{18}F]**T-401** was observed in the striatum, and frontal and occipital cortices in contrast with its lowest uptake in the brain stem. Regional time-activity curves were well described by a 2-tissue compartment model in consideration of the accumulation of a radiometabolite produced in the brain (2T+mCM). Metabolic analyses in mice also indicated the generation of a radiometabolite of [^{18}F]**T-401** in the brain, which supported the use of 2T+mCM for describing brain kinetics of [^{18}F]**T-401**. This model yielded reliable estimates of a total distribution volume (V_T) and the rank order of V_T (neocortex > striatum > cerebellum > thalamus > hippocampus > pons) was consistent with known regional expressions of MAGL. Pretreatment of monkeys with JW642 resulted in a dose-dependent reduction of [^{18}F]**T-401** retentions in the brain, and Lassen's graphical analysis of V_T enabled determination of plasma JW642 concentration (126.1 ng/mL) inducing 50% occupancy of central MAGL.

As shown above, I successfully developed [^{18}F]**T-401** as a novel, reversible, and selective PET radiotracer for MAGL. I also established an analytical method to quantify MAGL and its occupancy by an exogenous inhibitor in rhesus monkey brains using [^{18}F]**T-401**-PET. Based on its favorable imaging properties, [^{18}F]**T-401** has been advanced to further evaluation in humans (ID:UMIN000031787).

Publication

1. Hattori, Y.; Aoyama, K.; Maeda, J.; Arimura, N.; Takahashi, Y.; Sasaki, M.; Fujinaga, M.; Seki, C.; Nagai, Y.; Kawamura, K.; Yamasaki, T.; Zhang, M. R.; Higuchi, M.; Koike, T. Design, synthesis, and evaluation of (4R)-1-{3-[2-(^{18}F)fluoro-4-methylpyridin-3-yl]phenyl}-4-[4-(1,3-thiazol-2-ylcarbonyl)piperazin-1-yl]pyrr olidin-2-one ([^{18}F]**T-401**) as a novel positron-emission tomography imaging agent for monoacylglycerol lipase. *J. Med. Chem.* **2019**, *62*, 2362-2375.